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<b>(21) International Application Number:</b> PCT/JP98/00339 <b>(22) International Filing Date:</b> 28 January 1998 (28.01.98) <b>(30) Priority Data:</b> 9/15203 29 January 1997 (29.01.97) JP <b>(71) Applicant (for all designated States except US):</b> TAKEDA CHEMICAL INDUSTRIES, LTD. [JP/JP]; 1-1, Doshomachi 4-chome, Chuo-ku, Osaka-shi, Osaka 541 (JP). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> KAMEI, Shigeru [JP/JP]; 7-1-509, Sumiregaoka 1-chome, Takarazuka-shi, Hyogo 665 (JP). OHTA, Tsutomu [JP/JP]; 1-3, Satsukigaoka 5-chome, Ikeda-shi, Osaka 563 (JP). SAIKAWA, Akira [JP/JP]; 2-45, Nagaoka 2-chome, Nagaokakyo-shi, Kyoto 617 (JP). IGARI, Yasutaka [JP/JP]; 4-25-503, Motoyamam- inamimachi 5-chome, Higashinada-ku, Kobe-shi, Hyogo 658 (JP). <b>(74) Agents:</b> ASAHINA, Tadao et al.; Osaka Plant of Takeda Chemical Industries, Ltd., 17-85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka-shi, Osaka 532 (JP).		<b>(81) Designated States:</b> AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, GW, HU, ID, IL, IS, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TJ, TM, TR, TT, UA, US, UZ, VN, YU, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> SUSTAINED-RELEASE MICROSPHERES, THEIR PRODUCTION AND USE		
<b>(57) Abstract</b>  The present invention provides a method of producing a sustained-release microsphere which comprises emulsification, a physiological active peptide and a pamoic acid by a biodegradable polymer; a sustained-release microsphere comprising an about 0.01 to about 10 $\mu$ m particle size of a pamoic acid salt of physiologically active peptide and a biodegradable polymer; a sustained-release microsphere comprising a complex or a salt formed by a physiologically active peptide, a pamoic acid or a salt thereof and a biodegradable polymer; and a sustained-release preparation comprising the microsphere. The microsphere contains a large amount of the physiologically active peptide and can regulate a release rate of the physiological peptide.		

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DESCRIPTION

## Sustained-Release Microspheres, Their Production and Use

TECHNICAL FIELD

5       The present invention relates to sustained-release microspheres comprising a physiologically active peptide, a sustained-release preparation comprising the microspheres, and a method of producing the microspheres.

BACKGROUND ART

10       For the preparation of physiologically active peptides as sustained-release microspheres, various methods have been reported so far. For example, Japanese Patent Unexamined Publication No. 97334/1995 discloses a  
15       sustained-release preparation comprising a physiologically active peptide possessing LH-RH antagonist activity or a salt thereof and a biodegradable polymer having a free carboxyl group at one end, and a method of its production.

      Japanese Patent Unexamined Publication Nos.  
20       121222/1989 and 66625/1991 describe a control release drug composition comprising a water-insoluble adduct salt of a water-soluble peptide such as an LH-RH derivative converted by using a non-toxic water-insoluble acid such as pamoic acid, tannic acid or stearic acid, or the like, and a  
25       polymer like a polylactide or a copolymer of lactic acid and glycolic acid, and a method of its production, suggesting that drug release duration can be prolonged by converting the drug to water-insoluble, as defined to have a solubility in distilled water of not more than 25 mg/l.

30       Japanese Patent Unexamined Publication No. 68511/1991 describes a method of producing a sustained-release microparticle formulation wherein a microparticles is formed by dispersing a drug solution into a polymer solution in which the drug compound is insoluble, followed  
35       by hardening of the resulting product, and a microparticle formulation of somatostatin or a derivative thereof

obtained by the method. It also suggests the use of a pamoic acid salt may enable to stabilize the somatostatin derivative (octreotide) in the microparticle formulation.

Japanese Patent Unexamined Publication No. 221855/1993  
5 discloses a process for the production of a pharmaceutical composition for the sustained and controlled release of a peptide, obtained in the form of microsphere of a biodegradable polymeric material incorporating the peptide which comprises initially converting a water-soluble  
10 peptide into a water-insoluble peptide, followed by preparing an o/w emulsion, and extracting the organic solvent for the polymeric material in an excess of aqueous medium.

Furthermore, Japanese Patent Unexamined Publication  
15 No. 340543/1994 describes a sustained-release preparation wherein the embonic acid (pamoic acid) or ascorbic acid salt of a peptide as an active ingredient in a matrix of a polylactide having a lactide/glycolide molar ratio of 100:0 to 40:60, a molecular weight of 10,000 to 200,000, and a  
20 degree of polydispersion of 1.7 to 3.0, suggesting that embonic acid and ascorbic acid are useful as stabilizers in peptides in polylactides.

WO95/15767 describes the embonic acid salt (pamoic acid) of cetorelix (LH-RH antagonist) and a method of its  
25 production, stating that the duration of action was about the same as that of peptide embonate in a biologically degradable polymer.

As stated above, it has been known that a pamoic acid salt of a physiologically active peptide in a formulation  
30 enables to stabilize the physiologically active peptide or to control its release; however, there have been absolutely no reports of a composition wherein a fine and minute pamoic acid salt of a physiologically active peptide is formed in the presence of a biodegradable polymer, or a  
35 three-component salt comprising a physiologically active

peptide, a pamoic acid and a biodegradable polymer, and a composition containing it.

5 In addition, the sustained-release preparations obtained by these published methods are unsatisfactory in view of clinical application.

After extensive investigation aiming at resolving the above problems, the present inventors found that a sustained-release microsphere comprising a physiologically active peptide at high contents, and capable of controlling  
10 its release rate, can be produced by emulsification of a solution of a physiologically active peptide and a solution of a pamoic acid or a salt thereof by a biodegradable polymer.

More specifically, the present inventors found that a  
15 physiologically active peptide can be incorporated at high contents by emulsification of a physiologically active peptide having basic groups capable of forming salts with a pamoic acid, a pamoic acid or a salt thereof and a biodegradable polymer in a molecular dispersion like in  
20 solution to form a fine pamoic acid salt of the physiologically active peptide of about 0.01 to about 10  $\mu\text{m}$  in particle size not later than solvent removal, and producing a microsphere containing the pamoic acid salt, unlike conventional methods involves pre-conversion of  
25 physiologically active peptide to pamoic acid salt in the absence of polymer.

The present inventors also found in the case of a peptide having not less than 2 basic groups that a pamoic acid/physiologically active peptide ratio differing from  
30 that of microspheres produced by conventional methods which contain a previously prepared pamoic acid salt of a physiologically active peptide, and that the physiologically active peptide release rate can be controlled by the decomposition rate of the biodegradable  
35 polymer when the physiologically active peptide is allowed to form a complex or salt with both a pamoic acid and a

biodegradable polymer having a free carboxyl group, and a microsphere containing it is prepared.

After further investigations based on these findings, the inventors developed the present invention.

5

#### DISCLOSURE OF INVENTION

The present invention provides:

- (1) A method of producing a sustained-release microsphere which comprises emulsification of a physiologically active peptide or a salt thereof wherein the salt is not a pamoic acid salt and a pamoic acid or an alkaline metal salt thereof with a biodegradable polymer;
- (2) The method according to (1), which comprises emulsification of a solution of the physiologically active peptide or a salt thereof wherein the salt is not a pamoic acid salt and a solution of the pamoic acid or an alkaline metal salt thereof in solution of the biodegradable polymer with an organic solvent, and removing the solvent;
- (3) The method according to (1), which comprises dissolving the physiologically active peptide or a salt thereof wherein the salt is not a pamoic acid salt, the pamoic acid or an alkaline metal salt thereof and the biodegradable polymer in an organic solvent, and removing the solvent;
- (4) The method according to (1), which comprises emulsification of a solution of the physiologically active peptide or a salt thereof wherein the salt is not a pamoic acid salt and the biodegradable polymer with an organic solvent and a solution of the pamoic acid or an alkaline metal salt thereof, and removing the solvent;
- (5) The method according to (1), which comprises emulsification of a solution of the biodegradable polymer and the pamoic acid or an alkaline metal salt thereof with an organic solvent and a solution of the physiologically active peptide or a salt thereof wherein the salt is not a pamoic acid salt, and removing the solvent;

(6) The method according to any one of (2) to (5), wherein the removing of the solvent is conducted by in-water drying method;

5 (7) The method according to (6), which furthermore followed by freeze drying;

(8) The method according to any one of (2) to (5), wherein a concentration of the physiologically active peptide in the solution mixture is about 1 to about 25 wt% of the solution mixture;

10 (9) The method according to any one of (2) to (5), wherein a concentration of the biodegradable polymer in the solution mixture is about 1 to about 25 wt% of the solution mixture;

15 (10) The method according to any one of (2) to (5), wherein a concentration of the pamoic acid or a salt thereof in the solution mixture is about 0.05 to about 5 wt% of the solution mixture;

20 (11) The method according to (2) or (4), wherein the solution of the pamoic acid or a salt thereof is a methanol solution of the pamoic acid or a salt thereof;

25 (12) The method according to (4), wherein an amount of the solution of the pamoic acid or a salt thereof is about 2 to about 90 (v/v) % to the organic solvent of the physiologically active peptide and the biodegradable polymer in the of solution mixture;

(13) The method according to (1), wherein the physiologically active peptide or a salt thereof is a free base or a salt with a weak acid of not less than pKa4.0;

30 (14) The method according to (1), wherein the physiologically active peptide is a peptide having basic groups capable of forming salts with a pamoic acid;

35 (15) The method according to (1), wherein the physiologically active peptide is a peptide having not less than 2 basic groups capable of forming salts with a pamoic acid;

(16) The method according to (1), wherein the physiologically active peptide is an LH-RH agonist;

(17) The method according to (1), wherein the physiologically active peptide is an LH-RH antagonist;

5 (18) The method according to (1), the physiologically active peptide is a 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C<sub>2</sub>H<sub>5</sub> or a salt thereof;

(19) The method according to (1), the physiologically active peptide is a 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C<sub>2</sub>H<sub>5</sub> acetate;

10

(20) The method according to (1), wherein the biodegradable polymer is a polymer of  $\alpha$ -hydroxy carboxylic acids;

(21) The method according to (20), wherein the polymer of  $\alpha$ -hydroxy carboxylic acids is a lactic acid/glycolic acid polymer;

15

(22) The method according to (21), wherein a composition ratio of lactic acid/glycolic acid is 100/0 to 40/60 (mol%);

(23) The method according to (20), wherein a weight-average molecular weight of the biodegradable polymer is 3,000 to 100,000;

20

(24) The method according to (1), wherein the biodegradable polymer is a polylactic acid;

(25) The method according to (24), wherein a weight-average molecular weight of the biodegradable polymer is 10,000 to 60,000;

25

(26) The method according to any one of (2) to (5), wherein the organic solvent is a dichloromethane;

(27) The method according to (1), wherein the physiologically active peptide is a peptide having one basic group capable of forming a salt with a pamoic acid, and the sustained-release microsphere is a sustained-release microsphere comprising an about 0.01 to about 10  $\mu$ m particle size of a pamoic acid salt of the physiologically active peptide;

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(28) The method according to (1); wherein the physiologically active peptide is a peptide having not less than 2 basic groups capable of forming salts with a pamoic acid, and the sustained-release microsphere is a sustained-release microsphere comprising a complex or a salt formed by a physiologically active peptide, a pamoic acid or a salt thereof and a biodegradable polymer;

(29) A sustained-release microsphere which is obtainable by the method according to (1);

(30) The sustained-release microsphere which comprises an about 0.01 to about 10  $\mu\text{m}$  particle size of a pamoic acid salt of the physiologically active peptide and a biodegradable polymer;

(31) A sustained-release microsphere which comprises a complex or a salt formed by a physiologically active peptide, a pamoic acid or a salt thereof and a biodegradable polymer;

(32) A sustained-release microsphere which comprises not more than about 0.8 mol of pamoic acid to 1 mol of physiologically active peptide;

(33) The sustained-release microsphere according to (32), which comprises about 0.3 to about 0.7 mol of the pamoic acid to 1 mol of the physiologically active peptide is contained;

(34) The sustained-release microsphere according to any one of (29) to (32), wherein the physiologically active peptide is a physiologically active peptide having basic groups capable of forming salts with a weak acid of not less than  $\text{pK}_a 4.0$ ;

(35) The sustained-release microsphere according to any one of (29) to (32), wherein the physiologically active peptide is a peptide having basic groups capable of forming salts with a pamoic acid;

(36) The sustained-release microsphere according to any one of (29) to (32), wherein the physiologically active

peptide is a peptide having not less than 2 basic groups capable of forming salts with a pamoic acid;

(37) The sustained-release microsphere according to any one of (29) to (32), wherein the physiologically active peptide is an LH-RH agonist;

(38) The sustained-release microsphere according to any one of (29) to (32), wherein the physiologically active peptide is an LH-RH antagonist;

(39) The sustained-release microsphere according to any one of (29) to (32), wherein the physiologically active peptide is a 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C<sub>2</sub>H<sub>5</sub> or a salt thereof;

(40) The sustained-release microsphere according to any one of (29) to (32), wherein the physiologically active peptide is a 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C<sub>2</sub>H<sub>5</sub> acetate;

(41) The sustained-release microsphere according to (28) or (30), wherein the biodegradable polymer is a polymer of  $\alpha$ -hydroxy carboxylic acids;

(42) The sustained-release microsphere according to (41), wherein the polymer of  $\alpha$ -hydroxy carboxylic acids is a lactic acid/glycolic acid polymer;

(43) The sustained-release microsphere according to (42), wherein a composition ratio of lactic acid/glycolic acid is 100/0 to 40/60 (mol%);

(44) The sustained-release microsphere according to (41), wherein a weight-average molecular weight of the polymer is 3,000 to 100,000;

(45) The sustained-release microsphere according to any one of (29) to (32), wherein the biodegradable polymer is a polylactic acid;

(46) The sustained-release microsphere according to (45), wherein a weight-average molecular weight of the biodegradable polymer is 10,000 to 60,000;

(47) The sustained-release microsphere according to any one of (29) to (32), wherein a ratio of the

physiologically active peptide in the sustained-release microsphere is about 15 to about 85 wt% of the sustained-release microsphere;

5 (48) The sustained-release microsphere according to any one of (29) to (32), wherein a ratio of the pamoic acid or a salt thereof in the sustained-release microsphere is about 0.1 to about 25 wt% of the sustained-release microsphere;

10 (49) The sustained-release microsphere according to any one of (29) to (32), wherein a ratio of the biodegradable polymer in the sustained-release microsphere is about 15 to about 85 wt% of the sustained-release microsphere;

15 (50) The sustained-release microsphere according to (30), wherein a ratio of the about 0.01 to about 10  $\mu\text{m}$  particle size of a pamoic acid salt of the physiologically active peptide in the sustained-release microsphere is about 15 to about 90 wt% of the sustained-release microsphere;

20 (51) The sustained-release microsphere according to any one of (29) to (32), wherein the physiologically active peptide is 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C<sub>2</sub>H<sub>5</sub> or a salt thereof and a content of the peptide is about 15 to about 30 wt% to the total microcapsule;

25 (52) A sustained-release microsphere which is produced by the method according to (1);

(53) A sustained-release preparation which comprises the microsphere according to any one of (29) to (32);

30 (54) The sustained-release preparation according to (53), which is an injectable preparation;

(55) A sustained-release preparation which comprises the microsphere according to (37) or (38); and

35 (56) The sustained-release preparation according to (55), which is a treating or preventive agent for prostatic cancer, prostatic hypertrophy, endometriosis, hystero myoma,

dysmenorrhea, precocious puberty or breast cancer, or a contraceptive agent.

#### Detailed discription

5       The physiologically active peptides used in the method of the present invention may be any peptides which are capable of forming a salt with a pamoic acid and showing physiologically activities. Examples of the peptide are a peptide having about 300 to about 40,000, preferably about  
10   400 to 30,000, furthermore preferably about 500 to 20,000 molecular weight, and so on.

      Such peptides may preferably have basic groups which are capable of forming a salt with a weak acid of not less than pKa 4.0 (e.g. carbonic acid, bicarbonic acid, bornic  
15   acid, C<sub>1-3</sub> lower alkane-monocarbonic acid, etc.).

      When the physiologically active peptide has some basic groups in its molecule, so long as at least one basic group is capable of forming a salt with a pamoic acid, other groups may form salts. Physiologically active peptides  
20   having not only basic groups but also acidic groups which are free or forming salts may be involved in the physiologically active peptide of the present invention, so long as they are capable of forming salts with a pamoic acid.

25       The representative examples of activities of the physiologically peptides are hormone activity, etc.. The physiologically active peptides may be natural products, synthesized products, half-synthesized products or gene products, and furthermore may be analogs and/or derivatives  
30   thereof. The mechanism of these physiologically active peptides may be agonistic or antagonistic.

      Examples of the physiologically active peptides include luteinizing hormone-releasing hormone (sometimes referred to as LH-RH, gonadotropin-releasing hormone or Gn-  
35   RH), insulin, somatostatin, somatostatin derivative (Sandostatin; see US Patent Nos. 4,087,390, 4,093,574,

4,100,117 and 4,253,998), growth hormones (GH), growth hormone-releasing hormones (GH-RH), prolactin, erythropoietin (EPO), adrenocorticotrophic hormone (ACTH), ACTH derivatives (e.g., ebiratide), melanocyte-stimulating hormone (MSH), thyrotropin-releasing hormone (represented by the structural formula (Pyr)Glu-His-ProNH<sub>2</sub>, hereinafter also referred to as TRH) and salts and derivatives thereof (see Japanese Patent Unexamined Publication Nos. 121273/1975 and 116465/1977), thyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), vasopressin, vasopressin derivative (desmopressin, see Folia Endocrinologica Japonica, Vol. 54, No. 5, pp. 676-691 (1978)), oxytocin, calcitonin, glucagon, gastrin, secretin, pancreozymin, cholecystokinin, angiotensin, human placental lactogen, human chorionic gonadotropin (HCG), enkephalin, enkephalin derivatives (see US Patent No. 4,277,394 and European Patent Publication No. 31567), endorphin, kyotorphin, interferons (e.g.,  $\alpha$ -,  $\beta$ - and  $\gamma$ -interferons), interleukins (e.g., interleukin 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12), tuftsin, thymopoietin, thymosin, thymostimulin, thymic humoral factor (THF), blood thymic factor (FTS) and derivatives thereof (see US Patent No. 4,229,438), tumor necrosis factor (TNF), colony-stimulating factors (e.g., CSF, GCSF, GMCSF, MCSF), motilin, dynorphin, bombesin, neurotensin, caerulein, bradykinin, atrium sodium-excretion increasing factor, nerve growth factor (NGF), cell growth factors (e.g., EGF, TGF- $\alpha$ , TGF- $\beta$ , PDGF, acidic FGF, basic FGF), nerve nutrition factors (e.g., NT-3, NT-4, CNTF, GDNF, BDNF), and endothelin-antagonistic peptides and their analogs (derivatives) (see European Patent Publication Nos. 436189, 457195 and 496452, and Japanese Patent Unexamined Publication Nos. 94692/1991 and 130299/1991), a protein derived from  $\alpha$  1 domain of major histocompatibility class I antigen complex (Proceedings of the National Academy of Sciences of the United State of America, vol. 91,9086-9090

(1994) and vol. 94,11692-11697 (1997)) which has an activity of inhibiting an internalization of insulin receptor, insulin-like growth factor (IGF)-1 receptor, IGF-2 receptor, transferrin receptor, epidermal growth factor receptor, low density lipoprotein (LDL) receptor, macrophage scavenger receptor, GLUT-4 transporter, growth hormone receptor and leptin receptor, and their analogs (derivatives), furthermore their fragments or derivatives thereof.

When the physiologically active peptides are salts, the salts include pharmacologically acceptable salts. Examples of the salts are salts formed with inorganic acids (e.g., hydrochloric acid, sulfuric acid, nitric acid and boric acid) or salts formed with organic acids (e.g., carbonic acid, bicarbonic acid, succinic acid, acetic acid, propionic acid and trifluoroacetic acid), when the physiologically active peptide has a basic group such as the amino group. Examples of the salts are salts formed with inorganic bases (e.g., alkaline metals such as sodium and potassium, alkaline earth metals such as calcium and magnesium) or salts formed with organic base compounds (e.g., organic amines such as triethylamine, and basic amino acids such as arginine), when the physiologically active peptide has an acidic group such as the carboxy group. And, the physiologically active peptide may form a metal complex compound (e.g., copper complex compound, zinc complex compound). Provided that, a pamoic acid salt of the physiologically active peptide is excluded from a salt of the physiologically active peptide used as a material for the method of production of the present invention.

Preferable physiologically active peptides for the present invention include, for example, LH-RH analogues effective against diseases dependent on LH-RH or hormones induced thereby, such as prostatic cancer, prostatic hypertrophy, endometritis, hysterosarcoma, dysmenorrhea, precocious puberty and breast cancer, and as

contraceptives, and salts thereof, and somatostatin derivatives effective against diseases dependent on growth hormones and hormones induced thereby, and gastrointestinal diseases such as digestive ulcers, and salts thereof.

5        Specific examples of the LH-RH analogs or salts thereof are peptides described in Treatment with GnRH analogs: Controversies and perspectives, The Parthemon Publishing Group Ltd., 1996; and Japanese Patent Unexamined Publication Nos. 503165/1991, 101695/1991, 97334/1995 and  
10        259460/1996 and so on.

The preferable examples of the physiologically active peptide having LH-RH antagonistic activity are a physiologically active peptide represented by the formula:

15        
$$\text{X-D2Nal-D4ClPhe-D3Pal-Ser-A-B-Leu-C-Pro-DAlaNH}_2 \quad (\text{Ia})$$
 wherein X is N(4H2-furoyl)Gly or NAc, A is a residue selected from NMeTyr, Tyr, Aph(Atz) and NMeAph(Atz), B is a residue selected from DLys(Nic), DCit, DLys(AzaglyNic),  
20        DLys(AzaglyFur), DhArg(Et<sub>2</sub>), DAph(Atz) and DhCi, C is a residue selected from Lys(Nisp), Arg and hArg(Et<sub>2</sub>), and so on.

In addition, the preferable examples of the physiologically active peptide having LH-RH antagonistic activity are physiologically active peptides described in US Patent No. 5,580,957 and so on. These peptides can be  
25        prepared by the methods described in the above-mentioned references or publications or similar methods.

The preferable examples of the physiologically active peptide having LH-RH agonistic activity are a physiologically active peptide represented by the formula:

30        
$$\text{5-oxo-Pro-His-Trp-Ser-Tyr-Y-Leu-Arg-Pro-Z} \quad (\text{Ib})$$
 wherein Y is a residue selected from DLeu, DAla, DTrp, Dser(tBu), D2Nal and DHis(lmbZl), Z is NH-C<sub>2</sub>H<sub>5</sub> or Gly-NH<sub>2</sub>, and so on. Of these peptides, the peptide wherein Y is DLeu and Z is NH-C<sub>2</sub>H<sub>5</sub> is preferred. These peptides can be  
35        prepared by the methods described in the above-mentioned references or publications or similar methods.

Specific examples of the somatostatin derivatives or a salt thereof are described in Proceedings of National Academy of Science, USA, 93, 12513-12518, 1996 or references cited.

5 And, examples of the somatostatin derivatives which are selectively useful for cancer are

DPhe-Cys-Tyr-DTrp-Lys-Cys-ThrNH<sub>2</sub> (US Patent No. 5,480,870, European Patent Publication No. 50568) and so on.

10 Other preferable examples of the somatostatin derivatives are sandostatin (US Patent Nos. 4,087,390, 4,093,574, 4,100,117 and 4,253,998.) and so on. .

Preferable examples of the physiologically active peptides having one basic group capable of forming a salt with a pamoic acid are a physiologically active peptide or  
15 a salt thereof, represented by the formula [Ib] having a LH-RH agonistic activity and so on.

Preferable examples of the physiologically active peptides having not less than 2 basic groups capable of forming salts with a pamoic acid are a physiologically  
20 active peptide, or a salt thereof, represented by the formula (Ia) having a LH-RH antagonistic activity. And, the physiologically active peptide represented by the formula (Ib) can be also used.

25 Particularly, more preferably examples of the physiologically active peptide are 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C<sub>2</sub>H<sub>5</sub> or a salt thereof (particularly, acetate, etc.).

Abbreviations used in the present specification are  
30 defined as follows:

N(4H2-furoyl)Gly	: N-tetrafuoyl glycine residue
NAC	: N-acetyl group
D2Nal	: D-3-(2-naphtyl)alanine residue
D4ClPhe	: D-3-(4-chlorophenyl)alanine residue
35 D3Pal	: D-3-(3-pyridyl)alanine residue
NMeTyr	: N-methyl tyrosine residue

	Aph(Atz)	: N-[5'-(3'-amino-1'H-1',2',4'-triazolyl)]phenylalanine residue
	NMeAph(Atz)	: N-methyl-[5'-(3'-amino-1'H-1',2',4'-triazolyl)]phenylalanine residue
5	DLys(Nic)	: D-(epsilon-N-nicotinoyl)lysine residue
	DCit	: D-citrulline residue
	DLys(AzaglyNic)	: D-(azaglycyl nicotinoyl)lysine residue
	DLys(AzaglyFur)	: D-(azaglycyl furanyl)lysine residue
	DhArg(Et <sub>2</sub> )	: D-(N,N'-diethyl)homoarginine residue
10	DAph(Atz)	: D-N-[5'-(3'-amino-1'H-1',2',4'-triazolyl)]phenylalanine residue
	DhCi	: D-homocitrulline residue
	Lys(Nisp)	: (epsilon-N-isopropyl)lysine residue
	hArg(Et <sub>2</sub> )	: (N,N'-diethyl)homoarginine residue
15	DSer(tBu)	: D-(O-t-butyl)serine residue
	DHis(lmBzl)	: D-( $\pi$ -benzyl)histidine residue

Abbreviations for other amino acids are based on abbreviations specified by the IUPAC-IUB Commission on Biochemical Nomenclature (European Journal of Biochemistry, 138, 9-37, 1984) or abbreviations in common use in relevant fields. When an optical isomer may be present in amino acids, it is of the L-configuration, unless otherwise stated.

Examples of the biodegradable polymers used for the method of the present invention include homopolymers and copolymers, which are synthesized from one or more  $\alpha$ -hydroxy acids (e.g., glycolic acid, lactic acid, hydroxybutyric acid), hydroxydicarboxylic acids (e.g., malic acid), hydroxytricarboxylic acids (e.g., citric acid) etc., mixtures thereof; poly- $\alpha$ -cyanoacrylates; polyamino acids (e.g., poly- $\gamma$ -benzyl-L-glutamic acid) and maleic anhydride copolymers (e.g., styrene-maleic acid copolymers).

With respect to the above-described biodegradable polymer, copolymerization may be of the random, block or

graft type. When the above-mentioned  $\alpha$ -hydroxy acids, hydroxydicarboxylic acids and hydroxytricarboxylic acids have an optical active center in their molecular structures, they may be of the D-, L- or DL-configuration.

5 Of them, a lactic acid/glycolic acid polymer and a poly- $\alpha$ -cyanoacrylates are preferred, and furthermore a lactic acid/glycolic acid polymer is more preferred.

The biodegradable polymer is preferably (1) a biodegradable polymer consisting of a mixture of (A): a  
10 copolymer of a glycolic acid and a hydroxycarboxylic acid represented by the formula:



15 wherein R represents an alkyl group having 2 to 8 carbon atoms and (B): a polylactic acid or (2) a copolymer of lactic acid and glycolic acid.

With respect to the formula (II) above, the straight-chain or branched alkyl group represented by R, which has 2  
20 to 8 carbon atoms, is exemplified by ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, neopentyl, tert-pentyl, 1-ethylpropyl, hexyl, isohexyl, 1,1-dimethylbutyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl and 2-ethylbutyl. Preferably, a straight-  
25 chain or branched alkyl group having 2 to 5 carbon atoms is used. Such alkyl groups include ethyl, propyl, isopropyl, butyl and isobutyl. More preferably, R is ethyl.

The hydroxycarboxylic acid represented by the formula (II) is exemplified by 2-hydroxybutyric acid, 2-  
30 hydroxyvaleric acid, 2-hydroxy-3-methylbutyric acid, 2-hydroxycaproic acid, 2-hydroxyisocaproic acid and 2-hydroxycapric acid, with preference given to 2-hydroxybutyric acid, 2-hydroxyvaleric acid, 2-hydroxy-3-methylbutyric acid and 2-hydroxycaproic acid, with greater  
35 preference given to 2-hydroxybutyric acid. Although the hydroxycarboxylic acid may be of the D-, L- or D,L-

configuration, it is preferable to use a mixture of the D- and L-configurations wherein the ratio of the D-/L-configuration (mol%) preferably falls within the range from about 75/25 to about 25/75, more preferably from about 60/40 to about 40/60, and still more preferably from about 55/45 to about 45/55.

With respect to the copolymer of glycolic acid and a hydroxycarboxylic acid represented by the formula (II) (hereinafter referred to as glycolic acid copolymer), copolymerization may be of random, block or graft type. A random copolymer is preferred.

The hydroxycarboxylic acid represented by the formula (II) may be a mixture of one or more kinds in a given ratio.

With respect to the composition ratio of glycolic acid and the hydroxycarboxylic acid represented by the formula (II) in glycolic acid copolymer (A), it is preferable that glycolic acid account for about 10 to about 75 mol% and hydroxycarboxylic acid for the remaining portion. More preferably, glycolic acid accounts for about 20 to about 75 mol%, and still more preferably about 40 to about 70 mol%. The weight-average molecular weight of the glycolic acid copolymer is normally 2,000 to 100,000, preferably 3,000 to 80,000, and more preferably 5,000 to 50,000. The polydispersity (weight-average molecular weight/number-average molecular weight) of the glycolic acid copolymer is preferably about 1.2 to about 4.0, more preferably about 1.5 to about 3.5.

The above-described glycolic acid copolymer (A) can be produced by a known process, such as that described in Japanese Patent Unexamined Publication No. 28521/1986.

Although the above-described polylactic acid may be of the D- or L-configuration or a mixture thereof, it is preferable that the ratio of the D-/L-configuration (mol%) falls within the range from about 75/25 to about 20/80. The ratio of the D-/L-configuration (mol%) is more

preferably about 60/40 to about 25/75, and still more preferably about 55/45 to about 25/75. The weight-average molecular weight of said polylactic acid is preferably 1,500 to 100,000, more preferably 2,000 to 80,000, and  
5 still more preferably 10,000 to 60,000 (more preferably 15,000 to 50,000). Also, the dispersity of the polylactic acid is preferably about 1.2 to about 4.0, more preferably about 1.5 to about 3.5.

For producing a polylactic acid, two methods are  
10 known: ring-opening polymerization of lactide (a cyclic dimer of lactic acid) and polycondensation of lactic acid.

Glycolic acid copolymer (A) and polylactic acid (B) are used in a mixture wherein the (A)/(B) ratio (% by weight) falls within the range from about 10/90 to about  
15 90/10. The emulsification ratio (% by weight) is preferably about 20/80 to about 80/20, and more preferably about 30/70 to about 70/30.

If either component (A) or (B) is in excess, the preparation obtained shows a drug release pattern not much  
20 different from that obtained with the use of component (A) or (B) alone; the linear release pattern which is obtainable with the mixed matrices cannot be expected in the last stage of drug release. Although the decomposition/elimination rate of glycolic acid copolymer  
25 (A) and polylactic acid varies widely, depending on molecular weight or composition, drug release duration can be extended by increasing the molecular weight of polylactic acid mixed or lowering the emulsification ratio (A)/(B), since the decomposition/elimination rate of  
30 glycolic acid copolymer (A) is usually higher. Conversely, drug release duration can be shortened by decreasing the molecular weight of polylactic acid mixed or increasing the emulsification ratio (A)/(B). Drug release duration can also be adjusted by altering the kind and content ratio of  
35 hydroxycarboxylic acid represented by the formula (II).

When the biodegradable polymer used is a polylactic acid or lactic acid/glycolic acid polymer, its composition ratio (lactic acid/glycolic acid) (mol%) is about 100/0 to about 40/60, preferably about 100/0 to about 45/55, more preferably about 100/0 to about 50/50.

The weight-average molecular weight of the above-described lactic acid/glycolic acid polymer is preferably about 3,000 to about 100,000, more preferably about 5,000 to about 80,000.

The dispersity of the lactic acid/glycolic acid polymer is preferably about 1.2 to about 4.0, more preferably about 1.5 to about 3.5.

The decomposition/elimination rate of a lactic acid/glycolic acid polymer varies widely, depending on composition or molecular weight. Drug release duration can be extended by lowering the glycolic acid ratio or increasing the molecular weight, since decomposition/elimination is usually delayed as the glycolic acid ratio decreases. Conversely, drug release duration can be shortened by increasing the glycolic acid ratio or decreasing the molecular weight. To obtain a long-term (e.g., 1 to 6 months, preferably 1 to 4 months) sustained-release preparation, it is preferable to use a lactic acid/glycolic acid polymer whose composition ratio and weight-average molecular weight fall in the above-described ranges. With a lactic acid/glycolic acid polymer that decomposes more rapidly than that whose composition ratio and weight-average molecular weight fall in the above ranges, initial burst is difficult to suppress. On the contrary, with a lactic acid/glycolic acid polymer that decomposes more slowly than that whose composition ratio and weight-average molecular weight fall in the above ranges, it is likely that no effective amount of drug is released during some period.

Weight-average molecular weight, number-average molecular weight and dispersity, as defined herein, are

polystyrene-based molecular weights and dispersity determined by gel permeation chromatography (GPC) with 9 polystyrenes as reference substances with weight-average molecular weights of 120,000, 52,000, 22,000, 9,200, 5,050, 2,950, 1,050, 580 and 162, respectively. Measurements were taken using a GPC column KF804L  $\times$  2 (produced by Showa Denko) and an RI monitor L-3300 (produced by Hitachi, Ltd.), with chloroform as a mobile phase. Also, number-average molecular weight was calculated by dissolving the biodegradable polymer in an acetone-methanol mixed solvent, and titrating this solution with an alcoholic solution of potassium hydroxide with phenolphthalein as an indicator, to determine the terminal carboxyl group content. This molecular weight is hereinafter referred to as number-average molecular weight based on terminal group titration.

While the number-average molecular weight based on terminal group titration is an absolute value, the number-average molecular weight based on GPC measurement is a relative value that may vary depending on various analytical conditions (e.g., kind of mobile phase, kind of column, reference substance, slice width chosen, baseline chosen); it is therefore difficult to have an absolute numerical representation of the latter. In the case of a polymer having a free carboxyl group at one end, synthesized from lactic acid and glycolic acid by the catalyst-free polycondensation method, for example, however, the number-average molecular weight based on GPC measurement and that based on terminal group titration almost agree with each other. This fact for the lactic acid-glycolic acid polymer means that the number-average molecular weight based on terminal group titration falls within the range from about 0.5 to about 2 times, preferably from about 0.7 to about 1.5 times, the number-average molecular weight based on GPC measurement.

The lactic acid-glycolic acid polymer for the present invention can be produced by catalyst-free poly-

condensation from lactic acid and glycolic acid (Japanese Patent Unexamined Publication No. 28521/1986), or ring-opening polymerization from lactide, glycolide etc. using a catalyst (Encyclopedic Handbook of Biomaterials and Bioengineering Part A: Materials, Volume 2, Marcel Dekker, Inc., 1995). Although the polymer synthesized by ring-opening polymerization is usually a polymer having no carboxyl groups, a polymer obtained by chemically treating the above-described polymer to provide a terminal free carboxyl group (Journal of Controlled Release, Vol. 41, pp. 249-257, 1996) can also be used.

The above-described lactic acid-glycolic acid polymer having a free carboxyl group at one end can be readily produced by known methods (e.g., catalyst-free polycondensation method, see Japanese Patent Unexamined Publication No. 28521/1986), and a polymer having free carboxyl groups at unspecified position can be produced by known production methods (e.g., see WO94/15587 Publication).

Also, the lactic acid-glycolic acid polymer with a free carboxyl group at one end by chemical treatment after ring-opening polymerization is commercially available from Boehringer Ingelheim KG, for example.

Examples of the pamoic acid or a salt thereof may be a commercially available pamoic acid or a salt thereof. Examples of salts are alkaline metal salts (e.g. sodium salt, potassium salt, etc.), alkaline earth metal salts (e.g. calcium salt, magnesium salt, etc.), transition metal (e.g. zinc, iron, copper etc.) and so on. Particularly, alkaline metal salts such as sodium salt are preferred.

Examples of the solvents used for dissolution of the pamoic acid or a salt thereof and dissolution of the physiologically active peptide are water, alcohols (e.g. methanol, ethanol, etc.), pyridine, dimethylacetamide, acetic acid and so on. Preferable example is alcohols such as methanol and so on.

Examples of the organic solvents used for dissolution of the physiologically active peptide, pamoic acids or a salt and biodegradable polymer include halogenated hydrocarbons (e.g., dichloromethane, chloroform, 5 dichloroethane, trichloroethane, carbon tetrachloride, etc.), ethers (e.g., ethyl ether, isopropyl ether, etc.), fatty acid esters (e.g., ethylacetate, butylacetate), aromatic hydrocarbons (e.g., benzene, toluene, xylene, etc.) and alcohols (e.g. methanol, ethanol, etc.) with 10 preference given to halogenated hydrocarbons, particularly dichloromethane.

The production method of the present invention is characterized in producing a sustained-release microsphere comprising (i) an about 0.01 to about 10  $\mu\text{m}$  particle size 15 of a fine pamoic acid salt of the physiologically active peptide or (ii) a complex or salt formed by the physiologically active peptide, pamoic acid or a salt thereof and biodegradable polymer, wherein the fine pamoic acid salt and the complex or salt are formed by 20 emulsification of a physiologically active peptide and a pamoic acid or a salt thereof with a biodegradable polymer, without preforming a pamoic acid salt of the physiologically active peptide in the absence of a biodegradable polymer as conducted in the past methods.

25 Therefore, the mixing method of the physiologically active peptide, biodegradable polymer and pamoic acid or a salt thereof is not limited, so long as the pamoic acid salt is not formed in the absence of the biodegradable polymer.

30 Thus, the present invention specifically provides:  
(1) A method comprising emulsification of a solution of a physiologically active peptide or a salt thereof wherein the salt is not a pamoic acid salt and a solution of a pamoic acid or an alkaline metal salt thereof in a solution 35 of a biodegradable polymer with an organic solvent, and removing the solvent;

(2) A method comprising dissolving a physiologically active peptide or a salt thereof wherein the salt is not a pamoic acid salt, a pamoic acid or an alkaline metal salt thereof and a biodegradable polymer in an organic solvent, and removing the solvent;

(3) A method comprising emulsification of a solution of a physiologically active peptide or a salt thereof wherein the salt is not a pamoic acid salt and a biodegradable polymer with an organic solvent and a solution of a pamoic acid or an alkaline metal salt thereof, and removing the solvent; and

(4) A method comprising emulsification of solution of a biodegradable polymer and pamoic acid or an alkaline metal salt thereof with an organic solvent and a solution of a physiologically active peptide or a salt thereof wherein the salt is not a pamoic acid salt, and removing the solvent.

In the production method of the present invention, a concentration of the physiologically active peptide in the solution mixture is usually about 1 to about 25 wt%, preferably about 2 to about 20 wt% of the solution mixture.

A concentration of the biodegradable polymer in the solution mixture is usually about 1 to about 25 wt%, preferably about 2 to about 20 wt% of the solution mixture.

A concentration of the pamoic acid or a salt thereof in the solution mixture is usually about 0.05 to about 5 wt%, preferably about 0.2 to about 4 wt% of the solution mixture.

An amount of the solution of the pamoic acid or a salt thereof is usually about 2 to about 90 (v/v) % to the solution of the physiologically active peptide and the biodegradable polymer with an organic solvent.

For removing the solvent, in-water drying method, phase separation method and spray drying method are used.

The production method of the present invention is described specifically for each organic solvent removal method.

(I) In-water drying method:

5       A physiologically active peptide (including its salt) is added to solution of a biodegradable polymer with an organic solvent to yield a solution of the physiologically active peptide and the biodegradable polymer.

10       Examples of the organic solvent are halogenated hydrocarbons (e.g., dichloromethane, chloroform, dichloroethane, trichloroethane, carbon tetrachloride), ethers (e.g., ethyl ether, isopropyl ether), fatty acid esters (e.g., ethyl acetate, butyl acetate) and aromatic hydrocarbons (e.g., benzene, toluene, xylene). These  
15       solvents may be used in combination. The organic solvent used is preferably a halogenated hydrocarbon, more preferably dichloromethane.

20       Also, when a sufficient amount of physiologically active peptide is soluble in a solvent (e.g., water, alcohols (e.g., ethanol, methanol), acetonitrile, acetic acid) in a volume within 60% of the entire volume of the solution of the biodegradable polymer, a solution of the physiologically active peptide may be added to the solution of the biodegradable polymer to yield a solution of both,  
25       or the physiologically active peptide solution is emulsified in the solution of the biodegradable polymer to yield an o/o or w/o emulsion. In these procedures, it is undesirable to precipitate the physiologically active peptide.

30       Although the concentration of the biodegradable polymer used here in the solution varies, depending on the molecular weight of biodegradable polymer used, the kind of organic solvent, etc., it is normally chosen over the range from about 0.5 to about 70% (w/w), preferably about 1 to  
35       about 60% (w/w), and most preferably about 2 to about 50%

(w/w), when dichloromethane, for example, is used as the organic solvent.

The physiologically active peptide is normally added at about 30 mg to about 500 mg, preferably about 40 mg to about 400 mg, per ml of the above-described organic solvent in the biodegradable polymer solution.

Next, to the solution or o/o or w/o emulsion of the physiologically active peptide and biodegradable polymer, a solution of a pamoic acid or a pamoate (e.g., alkaline metal salts (sodium salt, potassium salt etc.), alkaline earth metal salts (e.g., calcium salt, magnesium salt) or salts with transition metals (e.g., zinc, iron, copper) (the solvent exemplified by water, alcohols (e.g., methanol, ethanol), pyridine, and dimethylacetamide) is added under emulsification by a known method such as the use of a homogenizer or ultrasonication.

Alternatively, when the physiologically active peptide or a salt thereof and the pamoic acid or a salt thereof, and furthermore the pamoic acid salt of the physiologically active peptide are completely soluble in an organic solvent (e.g., alcohols (methanol, ethanol etc.)), this organic solvent solution is added to the organic solvent solution of the biodegradable polymer under emulsification by a known method such as the use of a homogenizer or ultrasonication.

Although the pamoic acid or pamoate concentration in the solution in the above-described two addition methods is not subject to limitation, as long as it does not exceed the saturation concentration, it is preferably the saturation concentration, the ratio by volume of the pamoic acid or pamoate solution to the biodegradable polymer solution is preferably about 2 to about 90%, more preferably about 5 to about 70%, and most preferably about 10 to about 50%.

Next, the thus-obtained solution of the biodegradable polymer containing the physiologically active peptide and

pamoic acid (oil phase) is added to the second water phase to form an o (oil phase)/w (water phase) emulsion, after which the solvent in the oil phase is evaporated to yield microspheres. The volume of the water phase is normally  
5 chosen over the range from about 1 to about 10,000 times, preferably from about 2 to about 5,000 times, and most preferably from about 5 to about 2,000 times, that of the oil phase.

An emulsifier may be added to the above-described  
10 external water phase. The emulsifier may be any one, as long as it is capable of forming a stable o/w emulsion. Such emulsifiers include, for example, anionic surfactants (e.g., sodium oleate, sodium stearate, sodium lauryl sulfate), nonionic surfactants (e.g., polyoxyethylene  
15 sorbitan fatty acid esters (Tween 80, Tween 60, Atlas Powder Company), polyoxyethylene castor oil derivatives (e.g., HCO-60, HCO-50, Nikko Chemicals)), polyvinyl pyrrolidone, polyvinyl alcohol, carboxymethyl cellulose, lecithin, gelatin and hyaluronic acid. These emulsifiers  
20 may be used singly or in combination. The emulsifier is preferably used at concentrations within the range from about 0.01% to about 10% (w/w), more preferably from about 0.05% to about 5% (w/w).

An osmolarity regulator may be added to the above-  
25 described external water phase. The osmolarity regulator may be any one, as long as it provides an osmolarity when prepared as an aqueous solution.

Examples of the osmolarity regulators are polyhydric alcohols, monohydric alcohols, monosaccharides,  
30 disaccharides, oligosaccharides or derivatives thereof.

Such polyhydric alcohols include, for example, dihydric alcohols such as glycerol, pentahydric alcohols such as arabitol, xylitol and adonitol, and hexahydric alcohols such as mannitol, sorbitol and dulcitol. Of these  
35 alcohols, hexahydric alcohols are preferred, with greater preference given to mannitol.

Such monohydric alcohols include, for example, methanol, ethanol and isopropyl alcohol, with preference given to ethanol.

Such monosaccharides include, for example, pentoses  
5 such as arabinose, xylose, ribose and 2-deoxyribose, and hexoses such as glucose, fructose, galactose, mannose, sorbose, rhamnose and fucose, with preference given to hexoses.

Such oligosaccharides include, for example,  
10 trisaccharides such as maltotriose and raffinose, and tetrasaccharides such as stachyose, with preference given to trisaccharides.

Derivatives of such monosaccharides, disaccharides and oligosaccharides include, for example, glucosamine,  
15 galactosamine, glucuronic acid and galacturonic acid.

These osmolarity regulators may be used singly or in combination.

These osmolarity regulators are used at concentrations such that the osmolarity of the external water phase is  
20 about 1/50 to about 5 times, preferably about 1/25 to about 3 times, that of physiological saline.

Organic solvent removal can be achieved by known methods, including the method in which the organic solvent is evaporated under normal or gradually reduced pressure  
25 during stirring using a propeller stirrer, magnetic stirrer or the like, and the method in which the organic solvent is evaporated, while the degree of vacuum is adjusted.

The thus-obtained microspheres (also referred to as microcapsules) are collected by centrifugation or  
30 filtration, after they are repeatedly washed with several additions of distilled water to remove the physiologically active peptide, pamoic acid, drug support, emulsifier etc. adhering to the microsphere surface, again dispersed in distilled water etc. and freeze-dried.

35 To prevent mutual aggregation of particles during the production process, an anticoagulant may be added. The

anticoagulant is exemplified by water-soluble saccharides such as mannitol, lactose, glucose and starches (e.g., corn starch), and proteins such as glycine, fibrin and collagen. The anticoagulant is preferably mannitol.

5        Also, if necessary after freeze-drying, the microspheres may be heated under reduced pressure under conditions that do not cause their mutual fusion to remove the water and organic solvent therefrom. In this case, it is preferable that the microspheres be heated at a  
10       temperature slightly higher than the midpoint of glass transition temperature of the biodegradable polymer, as obtained using a differential scanning calorimeter when the temperature is elevated at a rate of 10 to 20°C per minute. More preferably, the microspheres are heated within the  
15       temperature range from the midpoint of glass transition temperature of the biodegradable polymer to a temperature higher by about 30°C than the glass transition temperature. When a lactic acid-glycolic acid polymer is used as the biodegradable polymer, in particular, the microspheres are  
20       heated within the temperature range from the midpoint of glass transition temperature to a temperature higher by 20°C than the glass transition temperature, preferably within the temperature range from the midpoint of glass transition temperature to a temperature higher by 10°C than  
25       the glass transition temperature.

Although heating time varies, depending on the amount of microspheres and other factors, it is generally preferable that heating time be about 12 to about 168 hours, more preferably about 48 to 120 hours after the  
30       microspheres reach a given temperature. Heating time is most preferably about 48 hours to about 96 hours.

Any heating method can be used, as long as microspheres are uniformly heated.

Preferable thermal drying methods include, for  
35       example, the method in which thermal drying is conducted in a thermostated chamber, fluidized bed chamber, mobile phase

or kiln, and the method using microwaves for thermal drying. Of these methods, the method in which thermal drying is conducted in a thermostated chamber is preferred.

(II) The phase separation method:

5 For producing microspheres by the phase separation method, a coacervating agent is gradually added to the oil phase described in the above (I) under stirring, to precipitate and solidify the biodegradable polymer. The volume of the coacervating agent is about 0.01 to about 10 1,000 times, preferably about 0.05 to about 500 times, more preferably about 0.1 to about 200 times to the volume of the oil phase.

Any coacervating agent can be used, as long as it is a polymeric, mineral oil or vegetable oil compound miscible 15 with the solvent for the biodegradable polymer and that does not dissolve the biodegradable polymer. Such coacervating agents include silicon oil, sesame oil, soybean oil, corn oil, cotton seed oil, coconut oil, linseed oil, mineral oil, n-hexane and n-heptane. These 20 may be used in combination of two or more kinds.

The thus-obtained microspheres are filtered to separate them, after which they are repeatedly washed with hexane, heptane etc. and heated to remove the coacervating agent. If necessary, in the same manner as with the above- 25 described in-water drying method, microspheres are washed with distilled water several times repeatedly to remove the free drug, drug-retaining substance etc. adhering to the microsphere surface.

(III) Spray drying method:

30 For producing microspheres by this method, the oil phase described in in-water drying method (I) above is sprayed via a nozzle into the drying chamber of a spray drier to volatilize the organic solvent in the fine droplets in a very short time, to yield microsphere. The 35 nozzle is exemplified by the double-fluid nozzle, pressure nozzle and rotary disc nozzle. The microspheres may be

then freeze-dried and thermally dried as necessary after being washed in the same manner as that described in in-water drying method (I).

For a dosage form other than the above-described microspheres, the oil phase described in in-water drying method (I) for microsphere production may be dried by evaporating the organic solvent and water, while the degree of vacuum is adjusted, followed by milling with a jet mill or the like to yield a fine powder.

The milled fine powder may be then freeze-dried and thermally dried after being washed in the same manner as that described in in-water drying method (I) for microsphere production.

The microspheres or fine powder can be orally or non-orally administered as such or in the form of various dosage forms prepared using them as a starting material. Specifically, they can be administered as muscular, subcutaneous, visceral and other injectable preparations or implant preparations, nasal, rectal, uterine and other transdermal preparations, oral preparations (e.g., solid preparations such as capsules (e.g., hard capsules, soft capsules), granules and powders; liquids such as syrups, emulsions and suspensions) etc.

For example, microspheres or a fine powder can be prepared as injectable formulations by suspending in water with a dispersing agent (e.g, surfactants such as Tween 80 and HCO-60, polysaccharides such as carboxymethyl cellulose and sodium alginate), a preservative (e.g., methyl paraben, propyl paraben), an isotonizing agent (e.g., sodium chloride, mannitol, sorbitol, glucose, proline) etc. to yield an aqueous suspension, or by dispersing in a vegetable oil such as sesame oil or corn oil to yield an oily suspension, whereby a practically useful sustained-release injectable preparation is obtained.

When the microspheres or fine powder is used in the form of an injectable suspension, their mean particle diameter is chosen over the range from about 0.1 to about 300  $\mu\text{m}$ , as long as the requirements concerning the degree of dispersion and needle passage are met. Preferably, the mean particle diameter is about 1 to about 150  $\mu\text{m}$ , more preferably about 2 to about 100  $\mu\text{m}$ .

The microspheres or fine powder can be prepared as a sterile preparation by such methods as the method in which the entire production process is aseptic, the method using gamma rays for sterilization, and the method in which a preservative is added, which methods are not to be construed as limitative.

The term microsphere, as defined herein, is any microparticle containing a physiologically active peptide and a biodegradable polymer. The microsphere is preferably nearly spherical. Such microparticles include, for example, microcapsules containing one drug core in each particle, a multiple-core microcapsule containing a large number of drug cores in each particle, and microparticles wherein a drug in the molecular form is dissolved or dispersed as a solid solution in a matrix.

The sustained-release microsphere of the present invention can be produced by the above-described production method of the present invention. For example, when a physiologically active peptide having basic groups capable of forming salts with a pamoic acid (particularly, a physiologically active peptide having the basic group) is used in the above production method, sustained-release microspheres comprising an about 0.01 to about 10  $\mu\text{m}$  particle size of fine pamoic acid salt of the physiologically active peptide and a biodegradable polymer can be produced, whereby a physiologically active peptide is incorporated in microspheres at higher efficiencies than in the conventional microsphere production method, wherein

a pamoic acid salt of a physiologically active peptide is formed in advance, then mixed with a biodegradable polymer to yield microspheres comprising the pamoic acid salt of the physiologically active peptide, to enable the  
5 production of microspheres containing a physiologically active peptide at high contents.

On the other hand, when using a physiologically active peptide having two or more basic groups capable of forming salts with a pamoic acid and a biodegradable polymer having  
10 a free carboxyl group, sustained-release microspheres comprising a similarly fine complex or salt formed with the physiologically active peptide, the pamoic acid or a salt thereof and the biodegradable polymer, can be produced. Here, the salt comprising three components may be any one,  
15 as long as the physiologically active peptide is incorporated between the pamoic acid and biodegradable polymer via a reversible bond, and may be a salt belonging to ortho salts, acidic salts, basic salts, complex salts, chelate salts etc. Complexes possibly formed by these  
20 three components are also included in the terminology of the above mentioned salts. The salt can be formed by emulsification of the three components in a molecular dispersion or similar state (e.g., solution state). In this case, the microsphere of the present invention, which  
25 contains the salts of three components, is characterized in that the pamoic acid/physiologically active peptide molar ratio therein is evidently smaller than that in the microspheres obtained by the conventional method; this demonstrates that the salt is a new salt differing from the  
30 salt in the conventional microsphere, which produces using a pamoic acid salt of a physiologically active peptide formed in the absence of a biodegradable polymer.

The sustained-release microspheres of the present invention contain a physiologically active peptide at high  
35 contents; the physiologically active peptide release from the preparation depends on the dissociation and dissolution

properties of the physiologically active peptide and/or a pamoic acid thereof, and the decomposition rate of the biodegradable polymer.

5           Thus, the present invention provides:

- (1) A sustained-release microsphere (A) which comprises an about 0.01 to about 10  $\mu\text{m}$  particle size of a pamoic acid salt of a physiologically active peptide and a biodegradable polymer; and
- 10       (2) A sustained-release microsphere (B) which comprises a complex or a salt formed by a physiologically active peptide, a pamoic acid or a salt thereof and a biodegradable polymer.

Each of the sustained-release microspheres (A) and (B)

15       is a sustained-release microsphere which comprises not more than about 0.8 mol, preferably about 0.1 to about 0.8 mol, more preferably 0.2 to 0.8 mol, furthermore preferably about 0.3 to 0.7 mol to 1 mol of the physiologically active peptide.

20           In the microsphere (A) of the present invention, examples of the physiologically active peptide, the pamoic acid or a salt thereof and the biodegradable polymer are same those as mentioned above.

Preferable examples of the physiologically active

25       peptide are a physiologically active peptide having groups capable of forming salts with a pamoic acid, particularly a physiologically active peptide having one basic group. And, preferable examples of the physiologically active peptide are LH-RH agonist represented by the formula (Ib),

30       and particularly a compound C: 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C<sub>2</sub>H<sub>5</sub> as shown in Example 11 as mentioned below, and so on.

Examples of the biodegradable polymer are a polylactic acid and a polymer of  $\alpha$ -hydroxy carboxylic acids, and

35       particularly a polylactic acid is preferred.

When the polylactic acid is used, the weight-average molecular weight is, for example, 10,000 to 60,000, more preferably 15,000 to 50,000.

5 As a composition ratio of lactic acid/glycolic acid is preferably 100/0 to 40/60 (mol%). Preferable weight-average molecular weight of the polymer is 5,000 to 80,000.

10 The particle size of the pamoic acid salt of the physiologically active peptide in the sustained-release microsphere (A) of the present invention is usually about 0.01 to about 10  $\mu\text{m}$ , preferably about 0.02 to about 5  $\mu\text{m}$ , more preferably about 0.02 to about 4  $\mu\text{m}$ .

15 In the sustained-release microsphere (A), the pamoic acid is usually included at the ratio of not more than about 0.8 mol, preferably about 0.1 to about 0.8 mol, more preferably about 0.2 to 0.8 mol, furthermore preferably about 0.3 to 0.7 mol to 1 mol of the physiological active peptide.

20 Although the emulsification ratio of the physiologically active peptide, the pamoic acid or a salt thereof and the biodegradable polymer in the sustained-release microsphere (A) may vary depending on kind of the physiologically active peptide, desired pharmacological action, duration of action and other factors, a ratio of the physiologically active peptide is usually not less than  
25 15 wt%, preferably about 15 to about 85 wt%, more preferably about 20 to about 80 wt%, furthermore preferably about 20 to about 50 wt% to the total microspheres. Particularly, when the physiologically active peptide is 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C<sub>2</sub>H<sub>5</sub> or a salt  
30 thereof (particularly, acetate), a content of the peptide is preferably about 15 to about 30 wt%.

35 A ratio of the pamoic acid or a salt thereof in the sustained-release microsphere (A) is usually about 0.1 to about 25 wt%, preferably about 0.5 to about 15 wt%, more preferably about 1 to about 10 wt% to the microsphere.

A ratio of the pamoic acid salt of the physiologically active peptide in the sustained-release microsphere (A) is usually about 15 (or about 15.1) to about 95 wt%, preferably about 20 to about 90 wt% to the sustained-release microspheres.

A ratio of the biodegradable polymer is usually about 15 to 85 wt%, preferably about 30 to about 60 wt% to the sustained-release microspheres.

In the microsphere (A) of the present invention, although the physiologically active peptides usually form salts with a pamoic acid, a part of the physiologically active peptides may exist without forming the salts.

In the microsphere (B) of the present invention, examples of the physiologically active peptide, the pamoic acid or a salt thereof and the biodegradable polymer are same those as mentioned above.

Preferable examples of the physiologically active peptide are a physiologically active peptide having not less than 2 basic groups capable of forming salts with a pamoic acid. Of these peptides, preferable examples of the physiologically active peptide are a LH-RH antagonist represented by the formula (Ia), and particularly a compound A as shown in Example 1 as mentioned below, and so on.

Examples of the biodegradable polymer are a polymer of  $\alpha$ -hydroxy carboxylic acids, and particularly a lactic acid/glycolic acid polymer is preferred. As a composition ratio of lactic acid/glycolic acid is preferably 100/0 to 40/60 (mol%). Preferable weight-average molecular weight of the polymer is 5,000 to 80,000.

The particle size of the pamoic acid salt of the physiologically active peptide in the sustained-release microsphere (B) of the present invention is usually about 0.01 to about 10  $\mu\text{m}$ , preferably about 0.02 to about 5  $\mu\text{m}$ , more preferably about 0.02 to about 4  $\mu\text{m}$ .

In the sustained-release microsphere (B), the pamoic acid is usually included at the ratio of not more than about 0.8 mol, preferably about 0.1 to about 0.8 mol, more preferably about 0.2 to 0.8 mol, furthermore preferably about 0.3 to 0.7 mol to 1 mol of the physiological active peptide.

Although the emulsification ratio of the physiologically active peptide, the pamoic acid or a salt thereof and the biodegradable polymer in the sustained-release microsphere (B) may vary depending on kind of the physiologically active peptide, desired pharmacological action, duration of action and other factors, a ratio of the physiologically active peptide is usually not less than 15 wt%, preferably about 15 to about 85 wt%, more preferably about 20 to about 80 wt%, furthermore preferably about 30 to about 80 wt%, for still more preferably about 40 to about 80wt% to the microspheres as sum of the physiologically active peptide, the pamoic acid or a salt thereof and the biodegradable polymer.

A ratio of the pamoic acid or a salt thereof in the microsphere (B) is usually about 0.1 to about 25 wt%, preferably about 0.5 to about 15 wt%, more preferably about 1 to about 10 wt% to the microspheres as sum of the physiologically active peptide, the pamoic acid or a salt thereof and the biodegradable polymer.

A ratio of the biodegradable polymer in the microsphere (B) is usually about 15 to about 85 wt%, preferably about 30 to about 60 wt% to the microspheres as sum of the physiologically active peptide, the pamoic acid or a salt thereof and the biodegradable polymer.

In the microsphere (B) of the present invention, although the physiologically active peptides usually form salts with a pamoic acid or a salt thereof and a biodegradable polymer, a part of the physiologically active peptides may exist without forming the salts.

The particle size of the pamoic acid salt of the physiologically active peptide can be determined by observing an oil phase in the way of preparing or a cross section of the microsphere with an optical microscope, or  
5 by observing a cross section of the microsphere with an electron microscope.

The sustained-release microsphere of the present invention is of low toxicity and can be used safely to human or mammals (e.g., monkey, bovines, pigs, dogs, cats,  
10 mice, rats, rabbits, etc.) as various sustained-release preparations.

Although varying depending on kind and content of a physiologically active peptides as an active ingredient, dosage form, duration of a physiologically active peptides  
15 release, subject diseases, subject animal species, and purpose of administration, the dose of the active ingredient of the microsphere preparation may be set at any level, as long as the active ingredient is effective. For example, when the sustained-release preparation is a one-  
20 month preparation, the dose of the physiologically active peptides per administration can be chosen as appropriate over the range from about 0.001 mg to about 100 mg, preferably from about 0.01 mg to about 50 mg more preferably about 0.05 mg to about 10 mg per adult (weight  
25 50 kg) in terms of the weight of microsphere.

More specifically, when the LH-RH antagonist represented by the general formula [Ia] above or the LH-RH agonist represented by the general formula [Ib] is used as the physiologically active peptide, it can be used as a  
30 treating or preventive agent for hormone-dependent diseases such as prostatic cancer, prostatic hypertrophy, endometritis, hysteromyoma, dysmenorrhea, metrofibroma, precocious puberty, breast cancer, gallbladder cancer, cervical cancer, chronic lymphatic leukemia, chronic  
35 myelocytic leukemia, colorectal cancer, gastritis, Hodgkin's disease, malignant melanoma, metastases, multiple

myeloma, non-Hodgkin lymphoma, non-small cell lung cancer, ovarian cancer, digestive ulcers, systemic fungal infections, small cell lung cancer, valvular disease of the heart, mastopathy, polycystic ovary, infertility, chronic  
5 anovulation, appropriately induced ovulation in women, acnes, amenorrhea (e.g., secondary amenorrhea), cystic diseases of the ovary and breast (including polycystic ovary), gynecologic cancers, ovarian hyperandrogenemia and hypertrichosis, AIDS due to T-cell production mediated by  
10 thymic blastogenesis, male contraception for treatment of and male sex criminals, as an agent for contraception and mitigation of symptoms of premenstrual syndrome (PMS), as a drug for *in vitro* fertilization (IVF), and for other  
purposes, especially as a treating or preventive agent for  
15 prostatic cancer, prostatic hypertrophy, endometritis, hystero myoma, metrofibroma, precocious puberty, breast cancer, etc., or an agent for contraceptive.

Although varying widely depending on dosage form, desired duration of drug release, target disease, subject  
20 animal species etc., the dose of the physiologically active peptide may be set at any level, as long as it is pharmacologically effective. The dose per administration of the drug can preferably be chosen as appropriate over the range from about 0.005 mg to about 10 mg/kg body weight  
25 per adult in the case of a 1-month sustained-release preparation. More preferably, it can be chosen as appropriate over the range from about 0.02 mg to about 5 mg/kg body weight.

The dose per administration of the microsphere in the  
30 sustained-release preparation can preferably be chosen as appropriate over the range from about 0.005 mg to 50 mg/kg body weight per adult. More preferably, it can be chosen as appropriate over the range from about 0.02 mg to 30 mg/kg body weight. Dosing frequency can be chosen as  
35 appropriate, e.g., once every several weeks, once every month, or once every several months, depending on kind and

content of active ingredient physiologically active peptide, dosage form, duration of physiologically active peptide release, target disease, subject animal species etc.

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[Mode of Working the Invention]

The present invention is hereinafter described in more detail by means of the following examples, comparative examples, and experimental examples, which are not to be construed as limitative, as long as they fall within the scope of the present invention. Unless otherwise specified, % means % by weight.

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Example 1

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A solution of 100 mg of pamoic acid in 2.7 ml of pyridine was added to a solution of 972 mg of N-(S)-2-tetrahydrofuroyl-Gly-D2Nal-D4ClPhe-D3Pal-Ser-NMeTyr-DLys(Nic)-Leu-Lys(Nisp)-Pro-DAlaNH<sub>2</sub> (herein after abbreviated to as Compound A) acetate (produced by TAP Company) and 1040 mg of lactic acid-glycolic acid copolymer (lactic acid/glycolic acid (molar ratio %) 50/50; weight-average molecular weight, 6,150; number-average molecular weight, 2,400; number-average molecular weight based on terminal group titration, 2,300; produced by Wako Pure Chemical) in 3 ml of dichloromethane. The mixture was emulsified using a small homogenizer for 60 seconds to yield S/O suspension (pamoic acid/Compound A (molar ratio), 0.5). After being cooled to 18°C, the suspension was poured into 400 ml of 0.1% aqueous solution of polyvinyl alcohol (EG-40, produced by Nippon Synthetic Chemical Industry Co., Ltd.) containing 5% mannitol, which had been previously adjusted at 18°C. The resultant mixture was prepared into S/O/W emulsion with the use of turbin-type homomixer at 7,000 rpm. The emulsion was stirred at room temperature for 3 hours to volatilize off the dichloromethane and solidify the oil phase, which was then

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collected by centrifugation with a centrifuge (05PR-22, Hitachi Ltd.) at 2,000 rpm. The resulting precipitate was again dispersed in distilled water, followed by centrifugation and removal of the separated free drug, etc.. After the collection microspheres were again dispersed in a small amount of distilled water and lyophilized to yield powdered microspheres. Encapsulation efficiency of Compound A into the microspheres was 90.2%. Content of Compound A and molar ratio of pamoic acid/Compound A in the microspheres were 38.6% and 0.49, respectively.

#### Example 2

Microspheres were obtained in similar manner to Example 1, except that lactic acid-glycolic acid copolymer was replaced by one (lactic acid/glycolic acid (molar ratio), 50/50; weight-average molecular weight, 10,100; number-average molecular weight, 3,720; number-average molecular weight based on terminal group titration, 3,500) and an amount of dichloromethane was 3.5 ml. Pamoic acid/Compound A (molar ratio) was 0.5. Encapsulation efficiency of Compound A into the microspheres was 91.8%. Content of Compound A and pamoic acid/Compound A in the microspheres were 39.2% and 0.51, respectively.

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#### Example 3

Microspheres were obtained in a similar manner to Example 1, except that lactic acid-glycolic acid copolymer was replaced by one (lactic acid/glycolic acid (molar ratio), 50/50; weight-average molecular weight, 12,700; number-average molecular weight; 4,780; number-average molecular weight based on terminal group titration, 4,900) and amount of dichloromethane was 3.8 ml. Added pamoic acid/Compound A (molar ratio) was 0.5. Encapsulation efficiency of Compound A into the microspheres was 89.9%.

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Content of Compound A and pamoic acid/Compound A in the microspheres were 38.4% and 0.53, respectively.

#### Example 4

5        Microspheres were obtained in a similar manner to Example 3, except the amount of pamoic acid and pyridine in Example 1 were changed to 200 mg and 5 ml, respectively. Added pamoic acid/Compound A (molar ratio) was 1.0. Encapsulation ratio of Compound A into the microspheres was  
10    94.1%. Content of Compound A and pamoic acid/Compound A in the microspheres were 38.3% and 0.63, respectively.

#### Example 5

15        A solution of 112 mg of disodium pamoic acid in 0.9 ml of distilled water was added to a solution of 972 mg of Compound A acetate (produced by TAP Company) and 1040 mg of lactic acid-glycolic acid copolymer (lactic acid/glycolic acid (molar %, 50/50); weight-average molecular weight, 12,700; number-average molecular weight, 4,780; number-  
20    average molecular weight based on terminal group titration, 4,900; produced by Wako Pure Chemical) in 4 ml of dichloromethane (pamoic acid/Compound A (molar ratio), 0.5). The mixture was emulsified using a small homogenizer for 60 seconds to yield S/O suspension (or W/O emulsion).  
25    After being cooled to 18°C, the suspension was poured into 400 ml of 0.1% aqueous solution of polyvinyl alcohol (EG-40, produced by Nippon Synthetic Chemical Industry Co., Ltd.) containing 5% mannitol, which had been previously adjusted at 18°C. The resultant mixture was prepared into  
30    S/O/W emulsion with the use of a turbin type homomixer at 7,000 rpm. The emulsion was stirred at room temperature for 3 hours to volatilize off the dichloromethane and solidify the oil phase, which was then collected by centrifugation with a centrifuge (05PR-22, Hitachi Ltd.) at  
35    2,000 rpm. The resulting precipitate was again dispersed in distilled water, followed by centrifugation and removal

of the separated free drug, etc.. After the collection microspheres were again dispersed in a small amount of distilled water and lyophilized to yield powdered microspheres. Encapsulation efficiency of Compound A into  
5 the microspheres was 89.8%. Content of Compound A and pamoic acid/Compound A (molar ratio) in the microspheres were 38.4% and 0.56, respectively.

#### Example 6

10        Microspheres were obtained in a similar manner to Example 5, except that the lactic acid-glycolic acid copolymer was replaced by one (lactic acid/glycolic acid (molar ratio %, 65/35); weight-average molecular weight, 12,500; number-average molecular weight, 4,170 and number-  
15 average molecular weight based on terminal group titration, 4,000) and the amount of dichloromethane was changed to 4.5 ml. Added pamoic acid/Compound A (molar ratio) was 0.5. Encapsulation ratio of Compound A into the microspheres was 89.6%. Content of Compound A and pamoic acid/Compound A  
20 (molar ratio) in the microspheres were 38.3% and 0.57, respectively.

#### Example 7

25        A solution of 0.45 g of disodium pamoic acid in 3.6 ml of distilled water was added to a solution of 4.06 g of Compound A acetate (produced by TAP Company) and 4 g of lactic acid-glycolic acid copolymer (lactic acid/glycolic acid=50/50 (mole %); weight-average molecular weight, 12,700; number-average molecular weight, 4,780; number-  
30 average molecular weight based on terminal group quantitation, 4,900; produced by Wako Pure Chemical) in 16 ml of dichloromethane (added pamoic acid/Compound A (molar ratio, 0.5)). The resulting mixture was emulsified using a small homogenizer for 60 seconds to yield S/O suspension  
35 (or W/O emulsion). After being cooled to 18°C, the resulting suspension was poured into 1600 ml of 0.1%

aqueous solution of polyvinylalcohol (EG-40, produced by Nippon Synthetic Chemical Industry Co., Ltd.) containing 5% mannitol, which had been previously adjusted at 18°C. The resulting mixture was prepared into S/O/W emulsion with the use of a turbine type homomixer at 7,000 rpm. The emulsion was stirred at room temperature for 3 hours to volatilize off the dichloromethane and to solidify the oil phase, which was then collected by centrifugation with a centrifuge (05PR-22, Hitachi Ltd.) at 2,000 rpm. The resulting precipitate was again dispersed in distilled water, followed by centrifugation and removal of the separated free drug, etc.. After the collection microspheres were again dispersed in a small amount of distilled water and lyophilized to yield powdered microspheres. The resulting microspheres were dried at 40°C for 96 hours under reduced pressure in an oven. Encapsulation efficiency of Compound A into the obtained microspheres (average diameter 22  $\mu$ m) was 93.8%. Content of Compound A and pamoic acid/Compound A (molar ratio) in the microspheres were 41.0% and 0.52, respectively.

#### Example 8

A solution of 0.244 g of disodium pamoic acid in 1.8 ml of distilled water was added to a solution of 2 g of NAc-D2Nal-D4ClPhe-D3Pal-Ser-NMeTyr-DLys(Nic)-Leu-Lys(Nisp)-Pro-DAlaNH<sub>2</sub> (herein after abbreviated to as Compound B) acetate (produced by TAP Company) and 2 g of lactic acid-glycolic acid copolymer (lactic acid/glycolic acid=50/50 (mole %); weight-average molecular weight, 12,700; number-average molecular weight, 4,780; number-average molecular weight based on terminal group titration, 4,900; produced by Wako Pure Chemical) in 9 ml of dichloromethane (pamoic acid/Compound B (molar ratio), 0.5, content of Compound B in its acetate was supposed as 86.7 %). The resulting mixture was emulsified using a small homogenizer for 60 seconds to yield S/O suspension (or W/O emulsion). After

being cooled to 18°C, the suspension was poured into 800 ml of 0.1% aqueous solution of polyvinyl alcohol (EG-40, produced by Nippon Synthetic Chemical Industry Co., Ltd.) containing 5% mannitol, which had been previously adjusted at 18°C. The resulting mixture was prepared into S/O/W emulsion with the use of a turbine-type homomixer at 7,000 rpm. The resulting emulsion was stirred at room temperature for 3 hours to volatilize off the dichloromethane and to solidify the oil phase, which was then collected by centrifugation with a centrifuge (05PR-22, Hitachi Ltd.) at 2,000 rpm. The resulting precipitate was again dispersed in distilled water, followed by centrifugation and removal of the separated free drug, etc.. After the collection microspheres were again dispersed in a small amount of distilled water and lyophilized to yield powdered microspheres. Encapsulation efficiency of Compound B into the microspheres was 98.9%. Content of Compound B and molar ratio of pamoic acid/Compound B in the microspheres were 43.6% and 0.52, respectively.

#### Example 9

A solution of 0.47 g of lactic acid-glycolic acid copolymer (lactic acid/glycolic acid (molar ratio %), 50/50); weight-average molecular weight, 18,700; number-average molecular weight, 6,180; number-average molecular weight based on terminal group titration, 6,000; produced by Wako Pure Chemical) in 15 ml of dichloromethane was added to a solution of 1.012 g of Compound A acetate (produced by TAP Company) and 0.112 g of disodium pamoic acid in 6 ml of methanol to prepare a homogeneous solution. From the solution the organic solvent was volatilized off by a rotary evaporator. The residue was sieved into particles of size of 75  $\mu$ m or smaller. The resulting fine powder was again dispersed in distilled water and centrifuged at 3,000 rpm. The separated drug, etc. was

removed. The collected fine powder was again dispersed in a small amount of distilled water and lyophilized to yield powder. Encapsulation efficiency of Compound A into the powder was 94.4%. Content of Compound A and pamoic acid/Compound A (mole ratio) in the microspheres were 56.5% and 0.60, respectively.

#### Example 10

A solution of 0.056 g of lactic acid-glycolic acid copolymer (lactic acid/glycolic acid (molar ratio), 50/50; weight-average molecular weight, 12,700; number-average molecular weight, 4,780; number-average molecular weight based on terminal group titration, 4,900; produced by Wako Pure Chemical) in 6 ml of dichloromethane, was added to a solution of 0.506 g of Compound A acetate (produced by TAP Company) and 0.056 g of disodium pamoic acid in 3 ml of methanol to prepare a homogeneous solution. From the solution the organic solvent was volatilized off by a rotary evaporator. The residue was sieved into particles of size of 75  $\mu\text{m}$  or smaller. The resulting fine powder was again dispersed in distilled water and centrifuged at 3,000 rpm. The separated drug, etc. was removed. The collected fine powder was again dispersed in a small amount of distilled water and lyophilized to yield powder. Encapsulation efficiency of Compound A in the powder was 94.6%. Content of Compound A and pamoic acid/Compound A (mole ratio) in the microspheres were 75.7% and 0.60, respectively.

#### Comparative Example 1

An aqueous solution of 9.4225 g of Compound A acetate was dropwisely added to a solution of 1.942 g of pamoic acid dissolved in an aqueous sodium hydroxide solution (added pamoic acid/Compound A (mole ratio)=1.0) under stirring to yield pamoic acid salt of Compound A as precipitate. The resulting precipitate was washed with

large excess of water and lyophilized. Each component of the lyophilized powder was measured by HPLC, and as a result, pamoic acid/Compound A (mole ratio) in the lyophilized powder was 1.08.

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#### Comparative Example 2

An aqueous solution of 54.03 mg of disodium pamoic acid was dropwisely added to an aqueous solution of 235.5 mg of Compound A acetate (added pamoic acid/Compound A (molar ratio), 1.0) under stirring to yield pamoic acid salt of Compound A as precipitate. The resulting precipitate was washed with large excess of water and lyophilized. Each component of the lyophilized powder was measured by HPLC, and as a result, pamoic acid/Compound A (molar ratio) in the lyophilized powder was 1.17.

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#### Comparative Example 3

An aqueous solution of 27.02 mg of disodium pamoic acid was dropwisely added to an aqueous solution of 245.2 mg of Compound A acetate (added pamoic acid/Compound A (mole ratio)=0.5) under stirring to yield pamoic acid salt of Compound A as precipitate. The resulting precipitate was washed with large excess of water and lyophilized. Each component of the lyophilized powder was measured by HPLC, and as a result, pamoic acid/Compound A (mole ratio) in the lyophilized powder was 1.26.

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#### Comparative Example 4

The pamoic acid salt of Compound A of Comparative Example 1 was ground. Using the salt of pamoic acid having an average particle size of 14  $\mu$ m, microspheres were prepared by the following procedure.

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Pamoic acid salt of Compound A (pamoic acid/Compound A (molar ratio)1.08) was added to a solution of 1.04 g of lactic acid-glycolic acid copolymer (lactic acid/glycolic acid (molar ratio), 50/50; average-weight molecular weight,

12,700; number-average molecular weight, 4,780 and number-average molecular weight based on terminal group titration, 4,500) in 4 ml of dichloromethane. The resultant mixture was emulsified with a small homogenizer to prepare S/O suspension. Using the suspension, microspheres were prepared in a similar manner to Example 1.

Encapsulation efficiency of Compound A into the microspheres was as low as 15%. Content of Compound A was 6.1%. From the fact that pamoic acid/Compound A (mole ratio) in the microspheres was the same as 1.12 of before encapsulation, Compound A was encapsuled as the salt of pamoic acid per se and is not considered to form a salt of copolymer of lactic acid-glycolic acid.

#### 15 Comparative Example 5

Microspheres were prepared in a similar manner to Example 3 except that pamoic acid and pyridine in Example 1 were not used. Encapsulation ratio of Compound A into the microspheres was 41.1%. Content of Compound A in the microspheres was 18.5%.

#### Comparative Example 6

An aqueous solution of 108.06 mg of disodium pamoic acid was dropwisely added to an aqueous solution of 221.4 mg of Compound B acetate (added pamoic acid/Compound B (molar ratio, 2.0) under stirring to yield pamoic acid salt of Compound B as precipitate. The resulting precipitate was washed with large excess of water and lyophilized. Each component of the lyophilized powder was measured by HPLC, and as a result, content of Compound B and pamoic acid/Compound B (mole ratio) in the lyophilized powder was 86.7% and 1.11, respectively.

#### Experiment 1

Microspheres produced in Examples 1-7 or pamoic acid salt of Compound A produced in Comparative Experiment 1

(sieved into particles of 25-75  $\mu\text{m}$ ) were used. About 6 mg of each of the particles were dispersed in 0.5 ml of a dispersant (distilled water dissolving 0.25 mg of carboxymethylcellulose, 0.5 mg of polysorbate 80 and 25 mg of mannitol). The dispersion was subcutaneously injected through 22 G needle into the back of male SD rats of 6-8 weeks. At specified intervals, rats were sacrificed, and microspheres or pamoic acid salts remained at the injected sites were collected for determination of Compound A, and the results are shown in Table 1.

Table 1

	1 week	2 weeks	3 weeks	4 weeks
Example 1	46%	22%	14%	10%
Example 2	56%	27%	16%	13%
Example 3	63%	35%	19%	12%
Example 4	51%	35%	20%	15%
Example 5	63%	33%	16%	9%
Example 6	63%	36%	21%	12%
Example 7	67%	28%	22%	5%
Ref.Ex. 1	40%	18%	13%	4%

## Example 11

An aqueous solution of 500 mg of 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C<sub>2</sub>H<sub>5</sub> acetate (produced by Takeda Chemical Industries, Ltd. Herein after abbreviated to as Compound C) in 0.45 ml of distilled water was added to a solution of 1,800 mg of polylactic acid (average-weight molecular weight, 50,000; number-average molecular weight, 25,000; produced by Taki Chemical) in 7.5 ml of dichloromethane. The resulting mixture was emulsified using a small homogenizer for 60 seconds to produce W/O emulsion, followed by addition of 0.85 ml of a solution of 85 mg of disodium pamoic acid dissolved in methanol. The resulting mixture was again emulsified using a small homogenizer for 60 seconds to yield S/O suspension. After

being cooled to 18°C, the suspension was poured into 400 ml of 0.1 % aqueous solution of polyvinyl alcohol (EG-40, Nippon Synthetic Chemical Industry Co., Ltd.) containing 5% of mannitol. Using a turbine-type homomixer, the mixture  
5 was prepared into S/O/W emulsion at 7,000 rpm. The resulting emulsion was stirred at room temperature for 3 hours to volatilize off dichloromethane and to solidify the oil phase, which was collected by centrifuge (5PR-22, Hitachi Ltd.) at 2,000 rpm. The resulting microspheres  
10 were dispersed in distilled water and further centrifuged, followed by removing the separated drug, etc.. The collected microspheres were again dispersed in a small amount of distilled water and lyophilized to yield powdered microspheres. Encapsulation efficiency of Compound C into  
15 the microspheres was 86.4%. Content of Compound C and pamoic acid/Compound A (mole ratio) in the microspheres were 18.2% and 0.50, respectively.

#### Example 12

20 Microspheres were prepared in a similar manner to Example 11, except that the polylactic acid was replaced by 1,500 mg of polylactic acid (weight-average molecular weight, 17,000; number-average molecular weight, 5,000; number-average molecular weight based on terminal group  
25 titration, 5,500; produced by Wako Pure Chemical), the amount of dichloromethane was changed to 8 ml and the methanol solution of disodium pamoic acid was replaced by 1.1 ml of a distilled water solution. Encapsulation efficiency of Compound C into the microspheres was 92.8%.  
30 Content of Compound C and pamoic acid/Compound C (mole ratio) in the microspheres were 21.9% and 0.78, respectively.

#### Example 13

35 An aqueous solution of 1,000 mg of Compound C dissolved in 0.9 ml of distilled water was added to a

solution of 3,600 mg of lactic acid (weight-average molecular weight, 24,300; number-average molecular weight, 7,790; number-average molecular weight based on terminal group titration, 8,000; produced by Wako Pure Chemical) in 8 ml of dichloromethane. The resulting mixture was emulsified using a small homogenizer for 60 seconds to yield W/O emulsion, followed by addition of a solution of 204 ml of disodium pamoic acid in 2 ml of methanol. The resulting mixture was again emulsified using a small homogenizer for 60 seconds to produce an almost clear but slightly opaque yellow solution. After being cooled to 18°C, the resulting yellow solution was poured into 800 ml of an aqueous solution of 0.1% polyvinyl alcohol (EG-40, produced by Nippon Synthetic Chemical) containing 5% mannitol. The resulting mixture was prepared into O/W emulsion by a turbine-type homomixer at 7,000 rpm. The resulting emulsion was stirred at room temperature for 3 hours to volatilize off the dichloromethane and to solidify the oil phase, which was collected by centrifuge (05PR-22, Hitachi Ltd.) at 2,000 rpm. The resulting microspheres were again dispersed in distilled water and centrifuged, followed by removing the separated drug, etc.. The collected microspheres were again dispersed in a small amount of distilled water and lyophilized to yield powdered microspheres. Encapsulation efficiency of Compound C into the microspheres (average diameter 25  $\mu$ m) was 100.0%. Content of Compound C and pamoic acid/Compound C (mole ratio) in the microspheres were 20.9% and 0.57, respectively.

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#### Example 14

Microspheres were prepared in a similar manner to Example 13 using yellow solution as prepared in Example 13, except that the polylactic acid was replaced by one (weight-average molecular weight, 40,000; number-average molecular weight, 26,700; produced by Taki Chemical) and

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the amount of dichloromethane was changed to 9 ml.  
Encapsulation efficiency of Compound C into the  
microspheres was 100.3%. Content of Compound C and pamoic  
acid/Compound C (mole ratio) in the microspheres were 21.0%  
5 and 0.56, respectively.

#### Example 15

Microspheres were prepared in a similar manner to  
Example 13 except that amounts of the polylactic acid,  
10 dichloromethane, disodium pamoic acid and methanol were  
changed to 5000 mg, 10 ml, 130 mg and 1.3 ml, respectively  
(the oil phase was not a solution observed in Example 13  
but a W/O emulsion). The resulting microspheres were  
further dried at 55°C for 120 hours under reduced pressure  
15 in an oven. Encapsulation efficiency of Compound C into  
the microspheres was 100.0%. Content of Compound C and  
pamoic acid/Compound C (mole ratio) in the microspheres  
were 16.4% and 0.39, respectively.

#### 20 Comparative Example 7

Microspheres were prepared in a similar manner to  
Example 11, except that the methanol solution of disodium  
pamoic acid was not added. Content of Compound C in the  
obtained microspheres was 7.7%.

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#### Comparative Example 8

An aqueous solution of 500 mg of disodium pamoic acid  
was dropwisely added to an aqueous solution of 2936.5 mg of  
Compound C (added pamoic acid/Compound C (molar ratio),  
30 0.5) under stirring to yield pamoic acid salt. The  
resulting salt was washed with large excess of water and  
lyophilized. By HPLC determination, pamoic acid/Compound C  
(molar ratio) in the lyophilized powder was 0.87. The  
powder was sieved to obtain particles of an average  
35 particle size of 10  $\mu$ m. The resulting powder were added  
to a solution of 1,800 mg of polyactic acid in 7.5 ml of

dichloromethane so that an amount of Compound C is equal to that in Example 11. Thus, microspheres were prepared using oil phase, wherein pamoic acid salt of Compound C was dispersed, by in-water drying method as a similar manner to  
5 Example 11, except that neither the aqueous solution of Compound C nor methanol solution of disodium pamoic acid was added. Content of Compound C in the obtained microspheres was 7.4%.

10 Comparative Example 9

Microspheres were prepared in a similar manner to Example 12, except that an aqueous solution of disodium pamoic acid in distilled water was not added. Content of Compound C in the obtained microspheres was 11.3%.

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INDUSTRIAL APPLICABILITY

The microsphere of the present invention contains a large amount of the physiologically active peptide and can regulate a release rate of the physiologically peptide.

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## CLAIMS

1. A method of producing a sustained-release microsphere which comprises emulsification of a physiologically active peptide or a salt thereof wherein said salt is not a pamoic acid salt and a pamoic acid or an alkaline metal salt thereof with a biodegradable polymer.

2. The method according to claim 1, which comprises emulsification of a solution of the physiologically active peptide or a salt thereof wherein said salt is not a pamoic acid salt and a solution of the pamoic acid or an alkaline metal salt thereof in a solution of the biodegradable polymer with an organic solvent, and removing the solvent.

3. The method according to claim 1, which comprises dissolving the physiologically active peptide or a salt thereof wherein said salt is not a pamoic acid salt, the pamoic acid or an alkaline metal salt thereof and the biodegradable polymer in an organic solvent, and removing the solvent.

4. The method according to claim 1, which comprises emulsification of a solution of the physiologically active peptide or a salt thereof wherein said salt is not a pamoic acid salt and the biodegradable polymer with an organic solvent and a solution of the pamoic acid or an alkaline metal salt thereof, and removing the solvent.

5. The method according to claim 1, which comprises emulsification of a solution of the biodegradable polymer and the pamoic acid or an alkaline metal salt thereof with an organic solvent and a solution of the physiologically active peptide or a salt thereof wherein said salt is not a pamoic acid salt, and removing the solvent.

6. The method according to any one of claims 2 to 5, wherein the removing of the solvent is conducted by in-water drying method.

7. The method according to claim 6, followed by freeze drying.

8. The method according to any one of claims 2 to 5, wherein a concentration of the physiologically active peptide in the solution mixture is about 1 to about 25 wt% of the solution mixture.

9. The method according to any one of claims 2 to 5, wherein a concentration of the biodegradable polymer in the solution mixture is about 1 to about 25 wt% of the solution mixture.

10. The method according to any one of claims 2 to 5, wherein a concentration of the pamoic acid or a salt thereof in the solution mixture is about 0.05 to about 5 wt% of the solution mixture.

11. The method according to claim 2 or 4, wherein the solution of the pamoic acid or a salt thereof is a methanol solution of the pamoic acid or a salt thereof.

12. The method according to claim 4, wherein an amount of the solution of the pamoic acid or a salt thereof is about 2 to about 90 (v/v) % to the organic solvent of the physiologically active peptide and the biodegradable polymer in the solution mixture.

13. The method according to claim 1, wherein the physiologically active peptide or a salt thereof is a free base or a salt with a weak acid of not less than pKa 4.0.

14. The method according to claim 1, wherein the physiologically active peptide is a peptide having basic groups capable of forming salts with a pamoic acid.

15. The method according to claim 1, wherein the physiologically active peptide is a peptide having not less than 2 basic groups capable of forming salts with a pamoic acid.

16. The method according to claim 1, wherein the physiologically active peptide is an LH-RH agonist.

17. The method according to claim 1, wherein the physiologically active peptide is an LH-RH antagonist.

18. The method according to claim 1, wherein the physiologically active peptide is a 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C<sub>2</sub>H<sub>5</sub> or a salt thereof.

19. The method according to claim 1, wherein the physiologically active peptide is a 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C<sub>2</sub>H<sub>5</sub> acetate.

20. The method according to claim 1, wherein the biodegradable polymer is a polymer of  $\alpha$ -hydroxy carboxylic acids.

21. The method according to claim 20, wherein the polymer of  $\alpha$ -hydroxy carboxylic acids is a lactic acid/glycolic acid polymer.

22. The method according to claim 21, wherein a composition ratio of lactic acid/glycolic acid is 100/0 to 40/60 (mol%).

23. The method according to claim 20, wherein a weight-average molecular weight of the biodegradable polymer is 3,000 to 100,000.

24. The method according to claim 1, wherein the biodegradable polymer is a polylactic acid.

25. The method according to claim 24, wherein a weight-average molecular weight of the biodegradable polymer is 10,000 to 60,000.

26. The method according to any one of claims 2 to 5, wherein the organic solvent is a dichloromethane.

27. The method according to claim 1, wherein the physiologically active peptide is a peptide having one basic group capable of forming a salt with a pamoic acid, and the sustained-release microsphere is a sustained-release microsphere comprising an about 0.01 to about 10  $\mu$ m particle size of a pamoic acid salt of the physiologically active peptide.

28. The method according to claim 1, wherein the physiologically active peptide is a peptide having not less than 2 basic groups capable of forming salts with a pamoic acid, and the sustained-release microsphere is a sustained-

release microsphere comprising a complex or a salt formed by a physiologically active peptide, a pamoic acid or a salt thereof and a biodegradable polymer.

29. A sustained-release microsphere which is obtainable by the method according to claim 1.

30. A sustained-release microsphere which comprises an about 0.01 to about 10  $\mu\text{m}$  particle size of a pamoic acid salt of the physiologically active peptide and a biodegradable polymer.

31. A sustained-release microsphere which comprises a complex or a salt formed by a physiologically active peptide, a pamoic acid or a salt thereof and a biodegradable polymer.

32. A sustained-release microsphere which comprises not more than about 0.8 mol of pamoic acid to 1 mol of physiologically active peptide.

33. The sustained-release microsphere according to claim 32, which comprises about 0.3 to about 0.7 mol of the pamoic acid to 1 mol of the physiologically active peptide.

34. The sustained-release microsphere according to any one of claims 29 to 32, wherein the physiologically active peptide is a physiologically active peptide having basic groups capable of forming salts with a weak acid of not less than  $\text{pKa}4.0$ .

35. The sustained-release microsphere according to any one of claims 29 to 32, wherein the physiologically active peptide is a peptide having basic groups capable of forming salts with a pamoic acid.

36. The sustained-release microsphere according to any one of claims 29 to 32, wherein the physiologically active peptide is a peptide having not less than 2 basic groups capable of forming salts with a pamoic acid.

37. The sustained-release microsphere according to any one of claims 29 to 32, wherein the physiologically active peptide is an LH-RH agonist.

38. The sustained-release microsphere according to any one of claims 29 to 32, wherein the physiologically active peptide is an LH-RH antagonist.

39. The sustained-release microsphere according to any one of claims 29 to 32, wherein the physiologically active peptide is a 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C<sub>2</sub>H<sub>5</sub> or a salt thereof.

40. The sustained-release microsphere according to any one of claims 29 to 32, wherein the physiologically active peptide is a 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C<sub>2</sub>H<sub>5</sub> acetate.

41. The sustained-release microsphere according to claim 29 or 31, wherein the biodegradable polymer is a polymer of  $\alpha$ -hydroxy carboxylic acids.

42. The sustained-release microsphere according to claim 41, wherein the polymer of  $\alpha$ -hydroxy carboxylic acids is a lactic acid/glycolic acid polymer.

43. The sustained-release microsphere according to claim 42, wherein a composition ratio of lactic acid/glycolic acid is 100/0 to 40/60 (mol%).

44. The sustained-release microsphere according to claim 41, wherein a weight-average molecular weight of the polymer is 3,000 to 100,000.

45. The sustained-release microsphere according to any one of claim 29 to 32, wherein the biodegradable polymer is a polylactic acid.

46. The sustained-release microsphere according to claim 45, wherein a weight-average molecular weight of the biodegradable polymer is 10,000 to 60,000.

47. The sustained-release microsphere according to any one of claims 29 to 32, wherein a ratio of the physiologically active peptide in the sustained-release microsphere is about 15 to about 85 wt% of the sustained-release microsphere.

48. The sustained-release microsphere according to any one of claims 29 to 32, wherein a ratio of the pamoic acid

or a salt thereof in the sustained-release microsphere is about 0.1 to about 25 wt% of the sustained-release microsphere.

49. The sustained-release microsphere according to any one of claims 29 to 32, wherein a ratio of the biodegradable polymer in the sustained-release microsphere is about 15 to about 85 wt% of the sustained-release microsphere.

50. The sustained-release microsphere according to claim 30, wherein a ratio of the about 0.01 to about 10  $\mu\text{m}$  particle size of a pamoic acid salt of the physiologically active peptide in the sustained-release microsphere is about 15 to about 90 wt% of the sustained-release microsphere.

51. The sustained-release microsphere according to any one of claims 29 to 32, wherein the physiologically active peptide is 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C<sub>2</sub>H<sub>5</sub> or a salt thereof and a content of the peptide is about 15 to about 30 wt% to the total microcapsule.

52. A sustained-release microsphere which is produced by the method according to claim 1.

53. A sustained-release preparation which comprises the microsphere according to any one of claims 29 to 32.

54. The sustained-release preparation according to claim 53, which is an injectable preparation.

55. A sustained-release preparation which comprises the microsphere according to claim 37 or 38.

56. The sustained-release preparation according to claim 55, which is a treating or preventive agent for prostatic cancer, prostatic hypertrophy, endometriosis, hystero myoma, dysmenorrhea, precocious puberty or breast cancer, or a contraceptive agent.

# INTERNATIONAL SEARCH REPORT

International Application No.

CT/JP 98/00339

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K9/16 A61K9/50 A61K47/12 A61K38/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, Y	GB 2 257 909 A (DEBIO RECH PHARMA SA) 27 January 1993 cited in the application see the whole document	29-31, 34-36, 41-56
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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# INTERNATIONAL SEARCH REPORT

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